

Supplemental Figures and Tables

Allele-specific control of replication timing and genome organization during development

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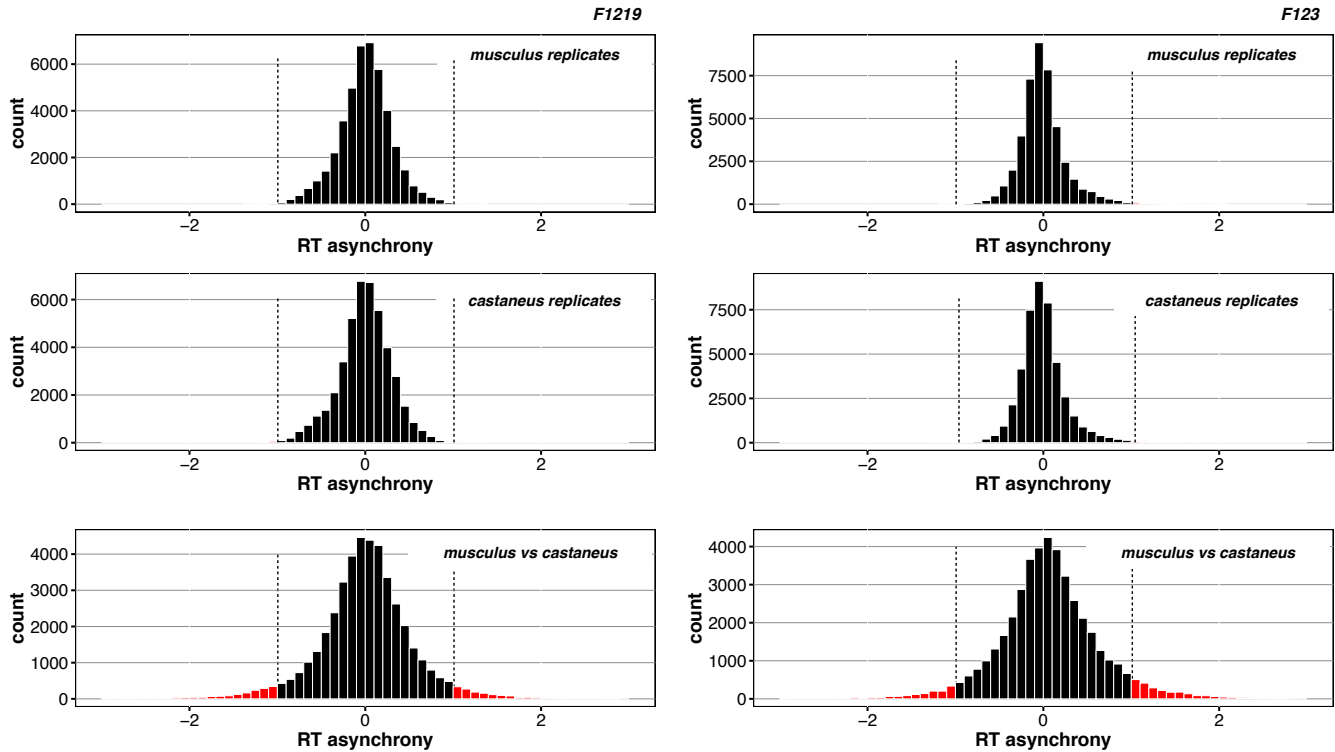
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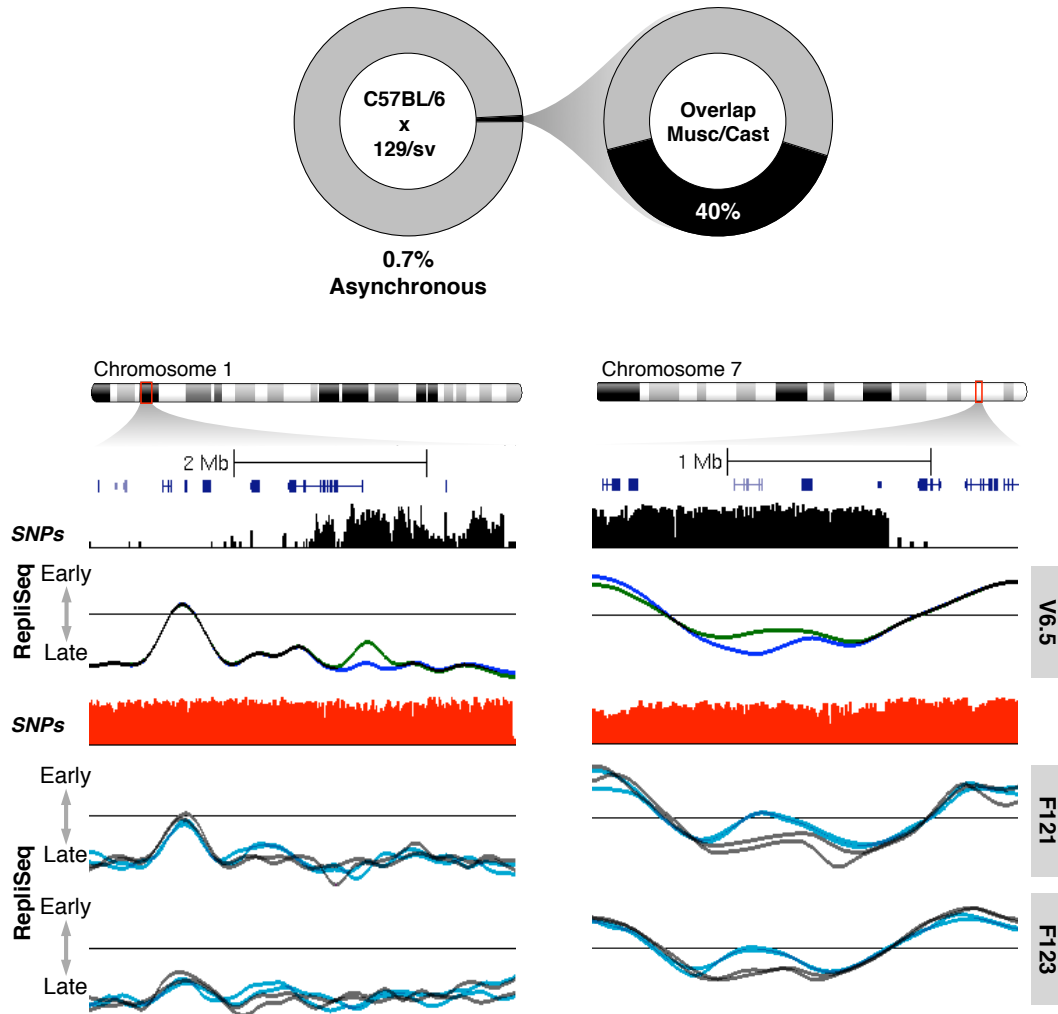
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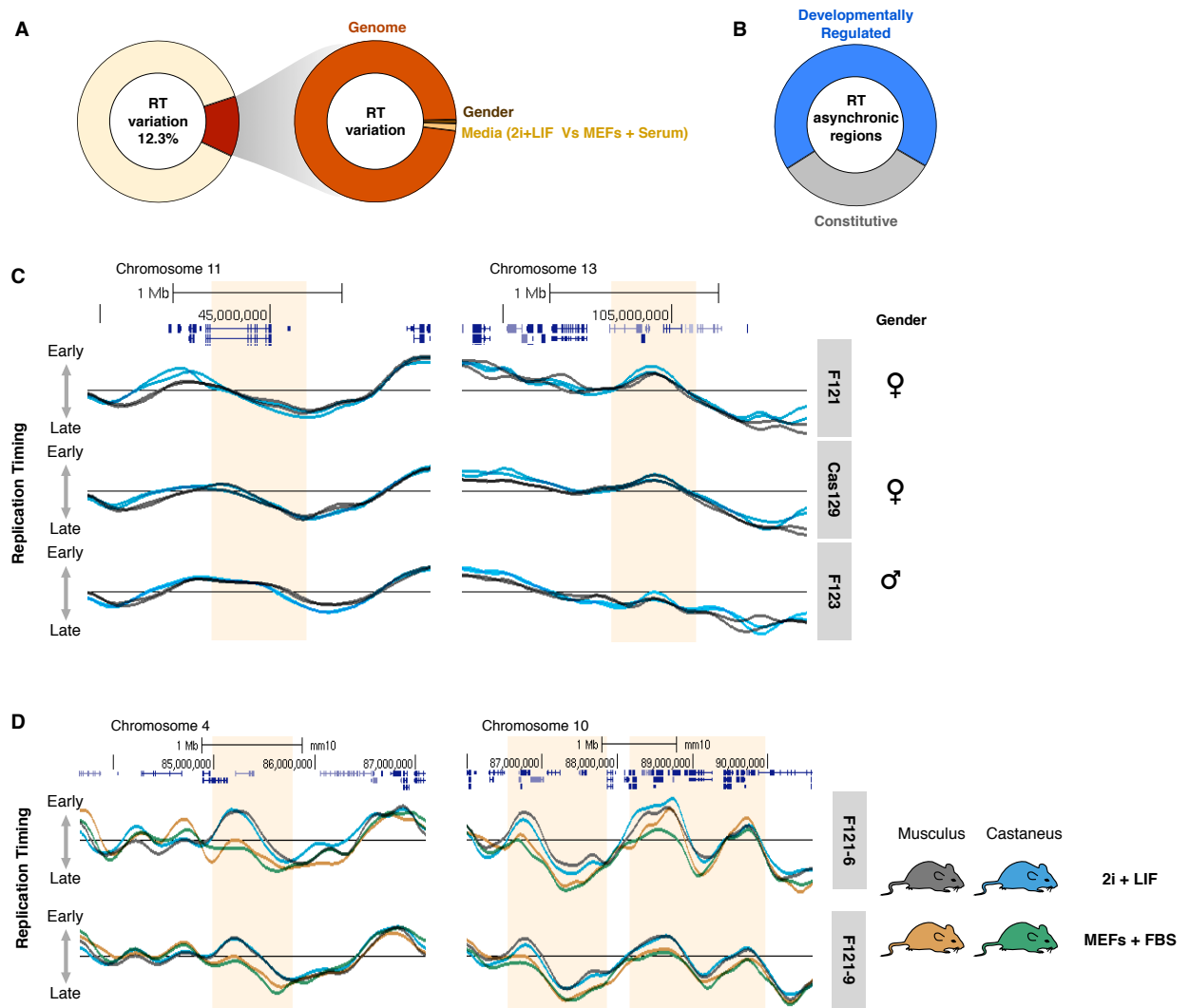
Supplemental Figure S1. Replication timing asynchrony in hybrid mouse ESCs *castaneus* x *musculus*.

RT asynchrony in *castaneus* x *musculus* mES cell lines. Histogram of RT differences between replicates of alleles of the same genome and comparison between distinct genomes are shown.



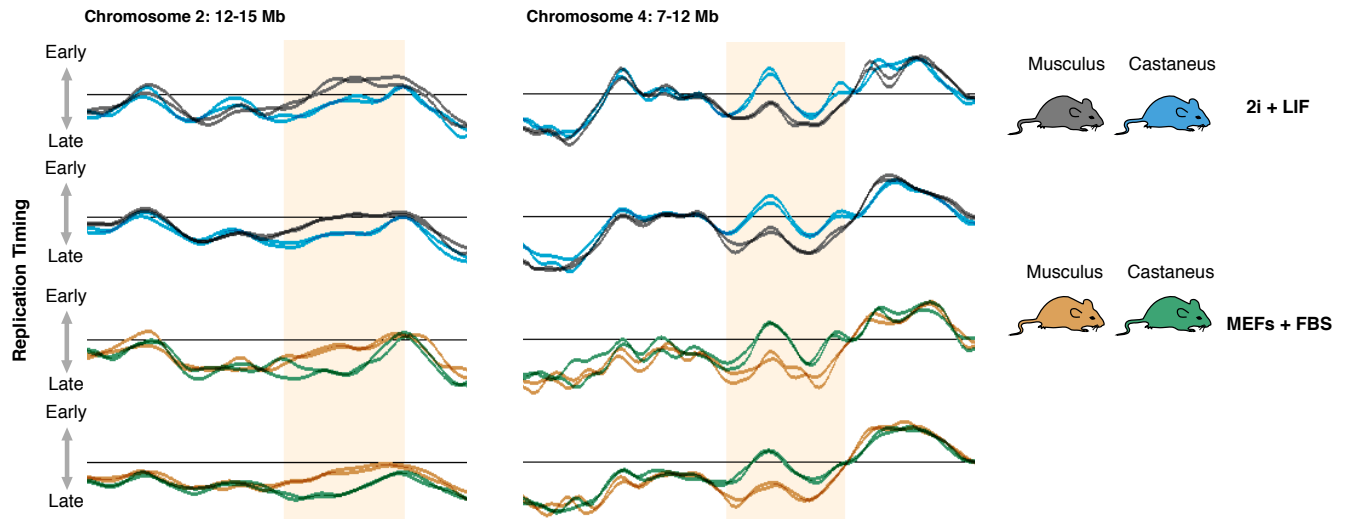
Supplemental Figure S2. Replication timing asynchrony in hybrid mouse ESCs derived from inbred strains crosses.

RT asynchrony in V6.5 cell line (cross between C57BL/6 and 129/sv strains) and the overlap with the RT asynchrony observed in *musculus* X *castaneus* crosses. Exemplary regions are shown at the bottom. RT profiles show exemplary asynchronous region that are specific for the V6.5 (left) or that overlap with differences *musculus* x *castaneus* crosses (right) .



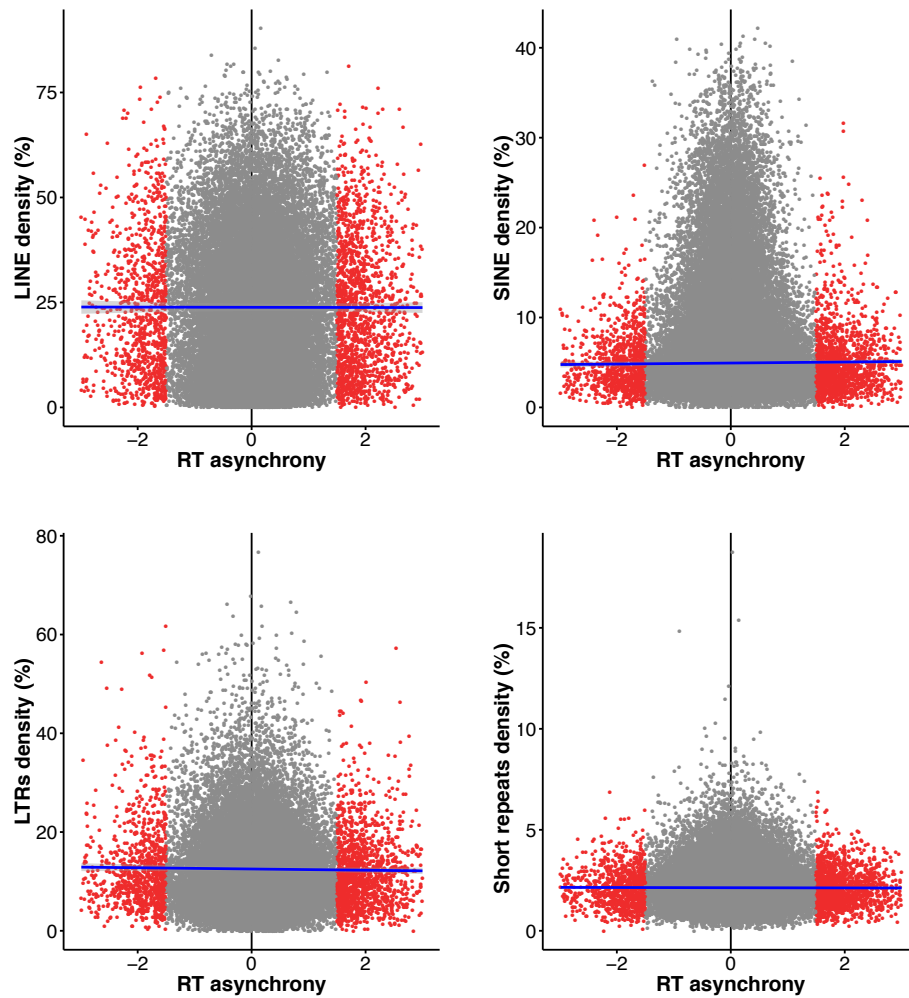
Supplemental Figure S3. Replication timing variation is linked to sub-species genomes.

(A) All RT variation across all cell lines was identified and sources quantified. 12% of the RT variation is associated to differences between sub-species genomes, 0.10% to gender differences and 0.17% to media conditions. No differences associated to parental configuration were found. (B) Asynchronously replicating domain in mESCs are enriched in genomic regions that are change RT during development. (C) Exemplary regions with differences between genders. Left RT profiles show a region that replicates earlier in male cell lines, while right RT profiles show a region that replicate earlier in females. (D) Exemplary regions showing RT differences linked to media conditions.



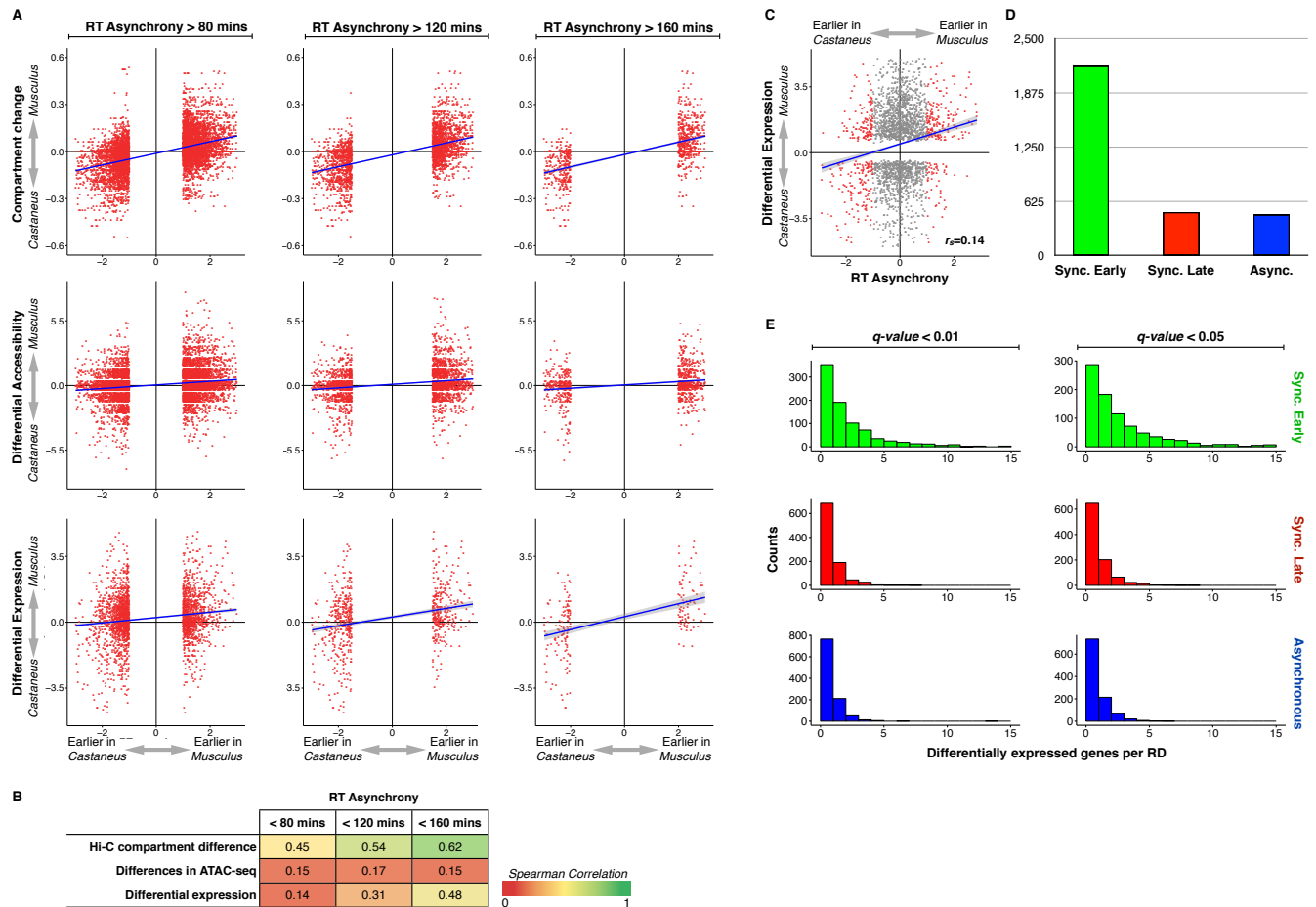
Supplemental Figure S4. Replication timing asynchrony is maintained in different growing conditions.

Exemplary RT asynchronous regions from two different mES cell lines cultured in different media conditions.



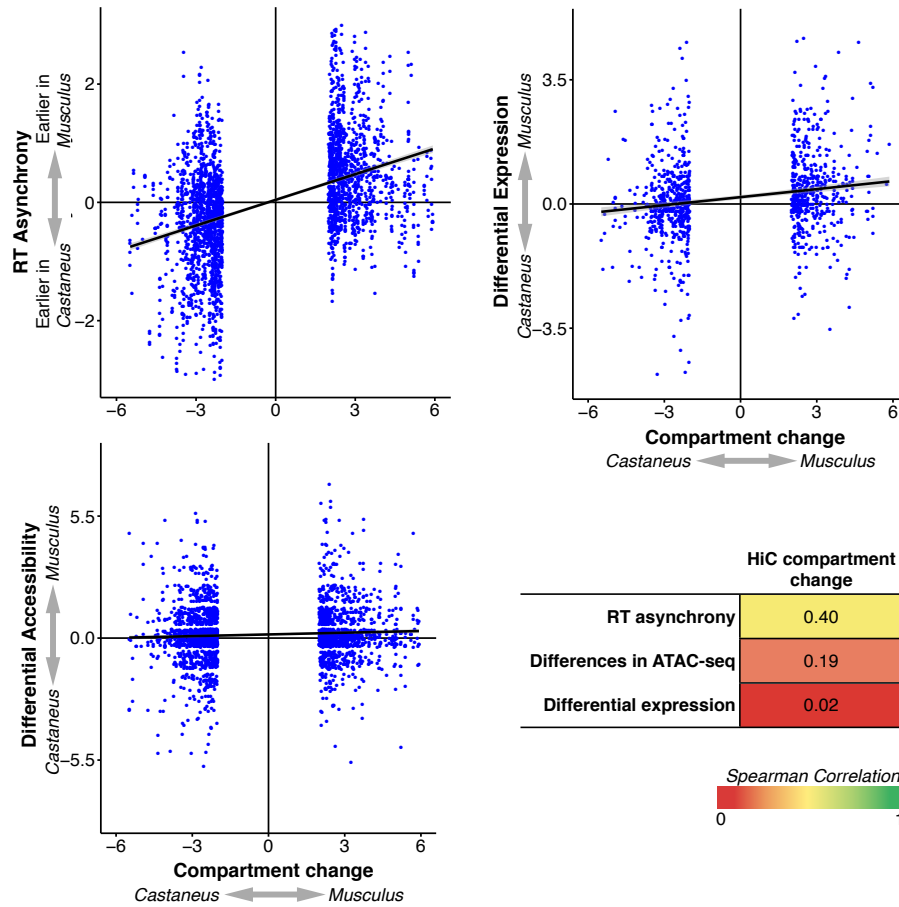
Supplemental Figure S5. Replication timing asynchrony is not linked to repetitive sequences.

Correlation analysis of RT asynchrony (RT differences between *musculus* and *castaneus* alleles) to LINEs, SINEs, LTRs and short repetitive sequences densities.



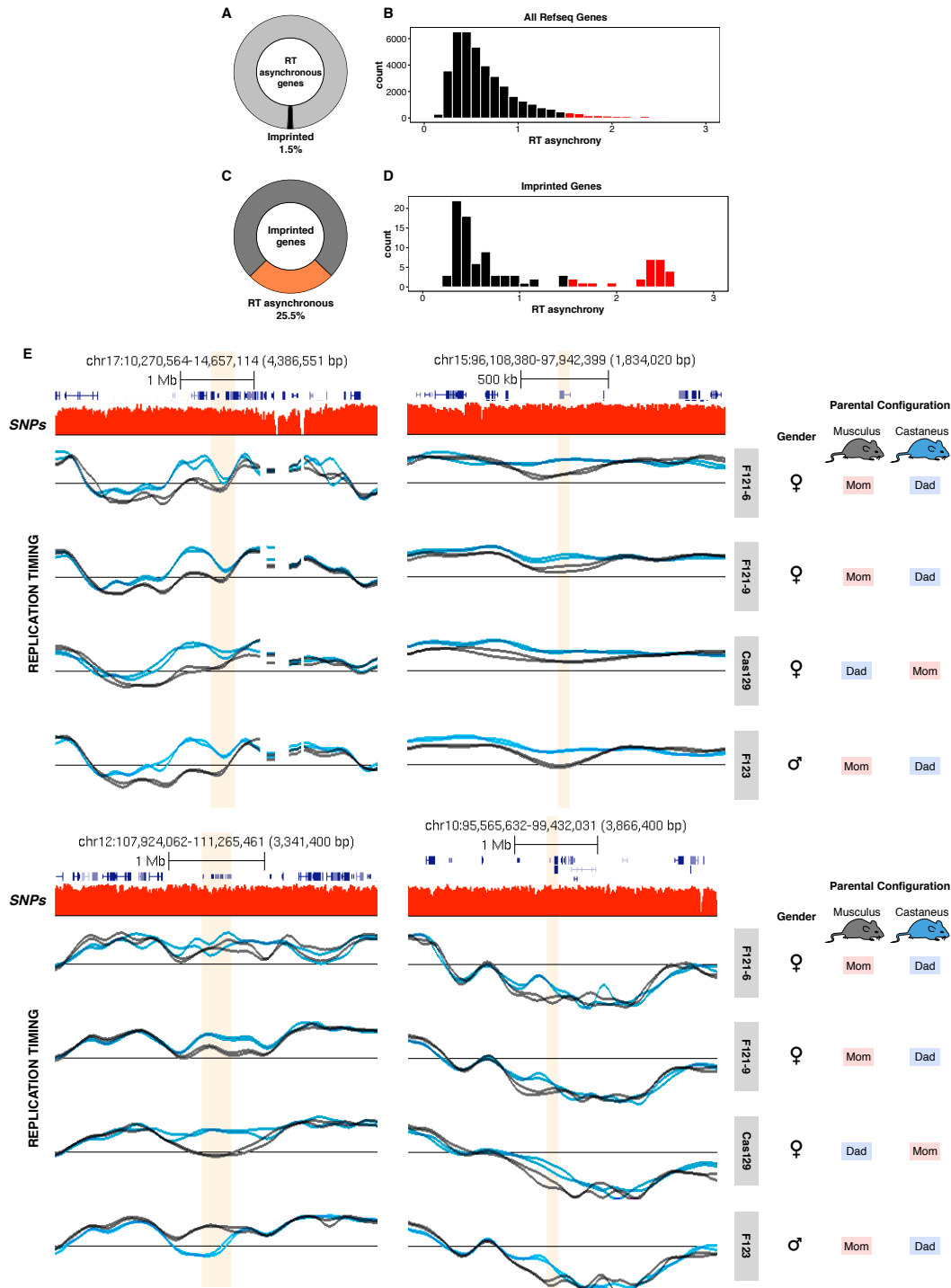
Supplemental Figure S6. Higher allelic RT differences strengthen the correlation of RT asynchrony and genome organization.

A) Correlation analysis of RT asynchrony to Hi-C compartment changes and differential accessibility and gene expression at distinct cutoffs (RT asynchrony was defined as differences in RT higher than 80, 120 and 160 minutes). B) Spearman correlation values of RT asynchrony and other genomic properties. C) Differentially expression is not associated to RT asynchrony. Differentially expressed genes were identified based on the FDR adjusted *p*-values (*q*-value) and correlated with the RT allelic differences. D) Differentially expressed genes distributions within synchronously (early or late replicating) and asynchronously replicating domains. E) Most asynchronously replicating domains do not contain differentially expressed genes. Histogram distributions of differentially expressed genes in synchronously (early or late replicating) and asynchronously replicating domains.



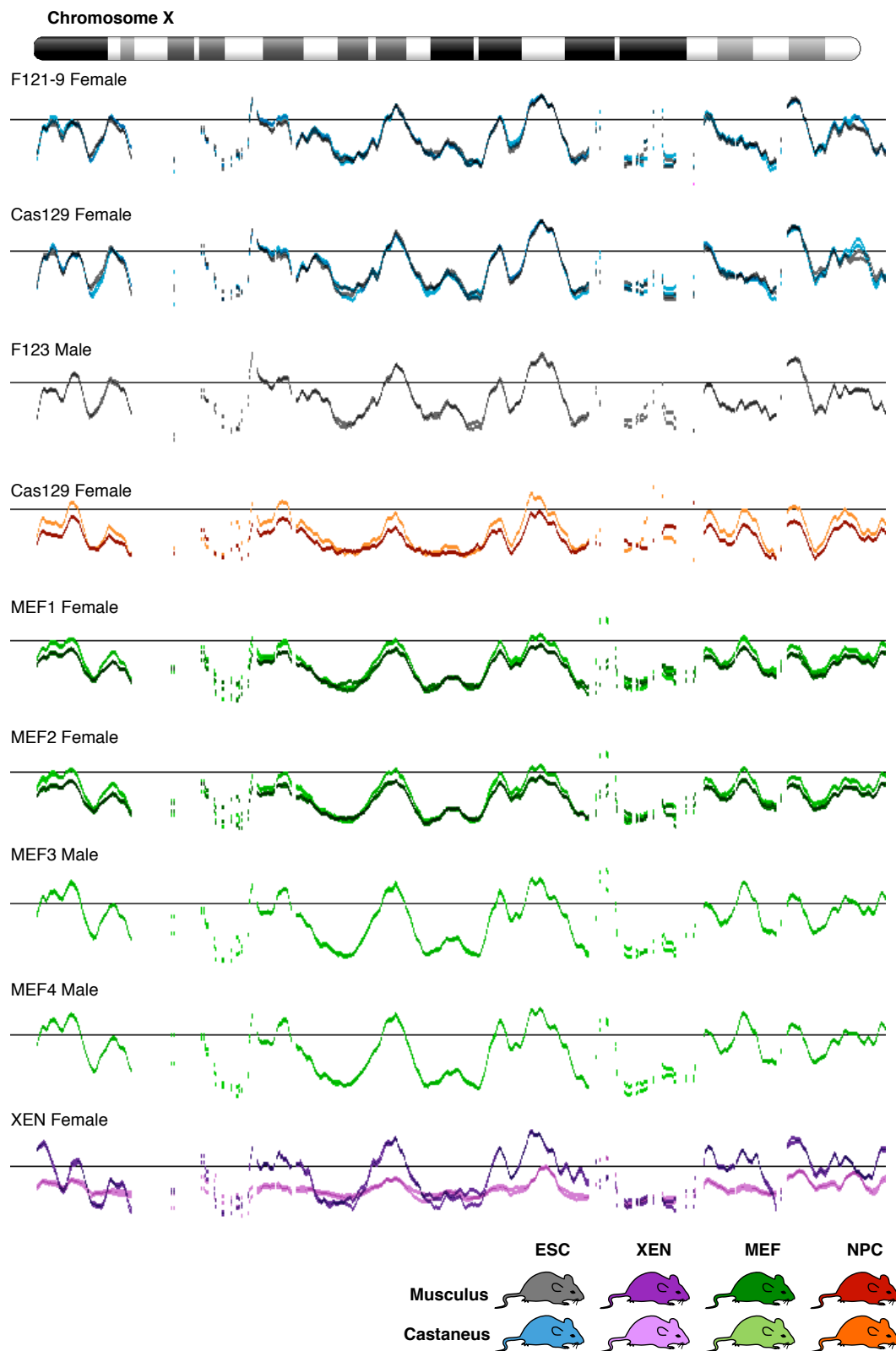
Supplemental Figure S7. Changes in 3D genome organization correlate with RT asynchrony but not with gene expression or chromatin accessibility.

Scatter plots of Hi-C compartment changes vs. RT asynchrony, differences in chromatin accessibility and differential expression. Spearman correlation values of Hi-C compartment changes vs. RT asynchrony, differences in chromatin accessibility and differential expression.

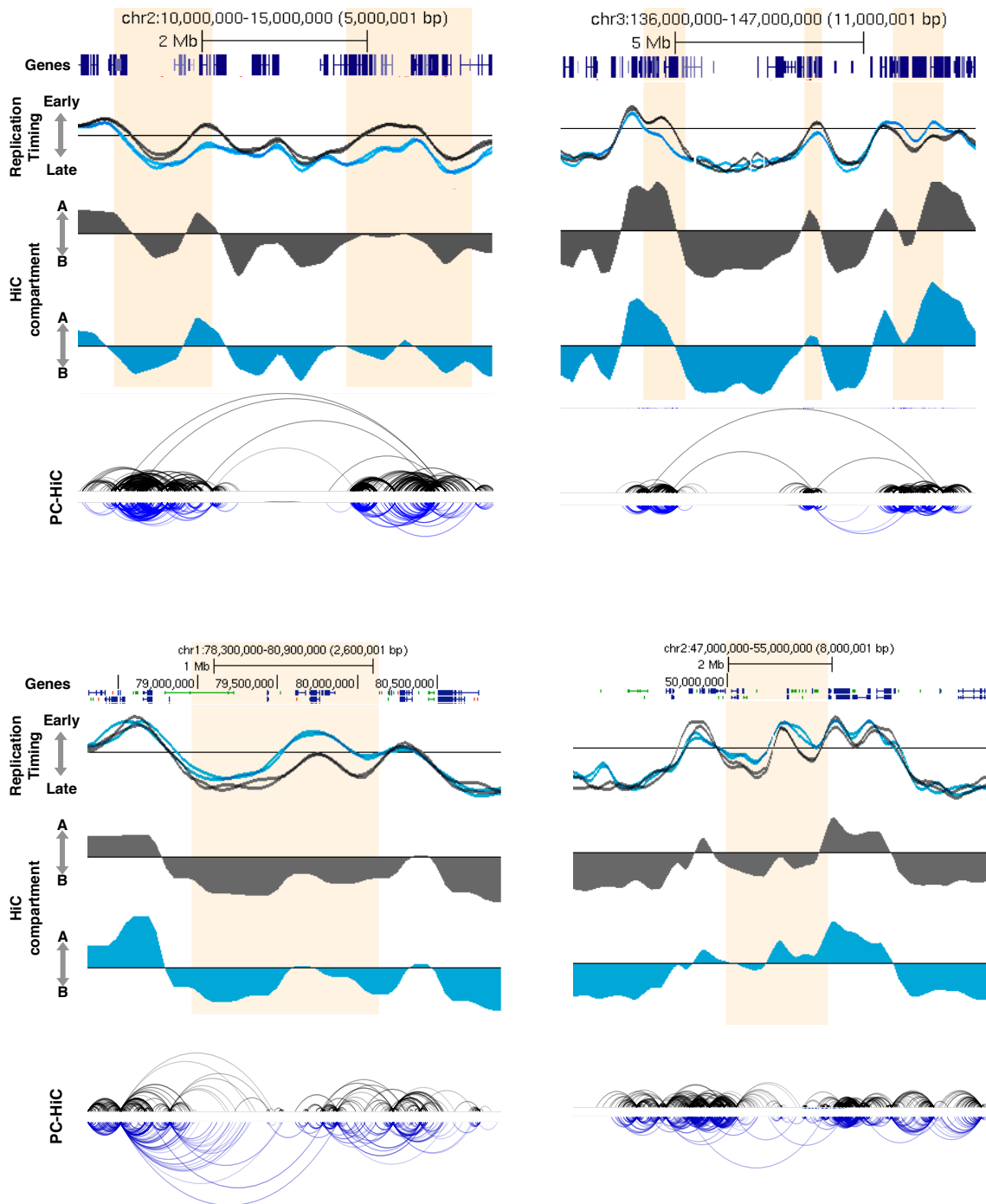


Supplemental Figure S8. RT asynchrony is not associated to gene imprinting.

A) RT asynchrony is not enriched at imprinted genes in hybrid mESCs. RT values at the transcription start sites (TSS) of all RefSeq genes were obtained and asynchronous genes identified. Only 1.5% of the RT asynchronous genes are imprinted. B) RT asynchrony across all RefSeq genes. C) Only a small fraction (25.5%) of imprinted genes replicate asynchronously in hybrid mESCs. D) RT asynchrony in imprinted genes. E) Exemplary RT profiles at the imprinted genes with the highest RT differences. Imprinted genes are highlighted in each plot. RT differences are linked to the respective genomes rather than imprinting. List of imprinted genes was extracted from the imprinted genes database: www.geneimprint.com

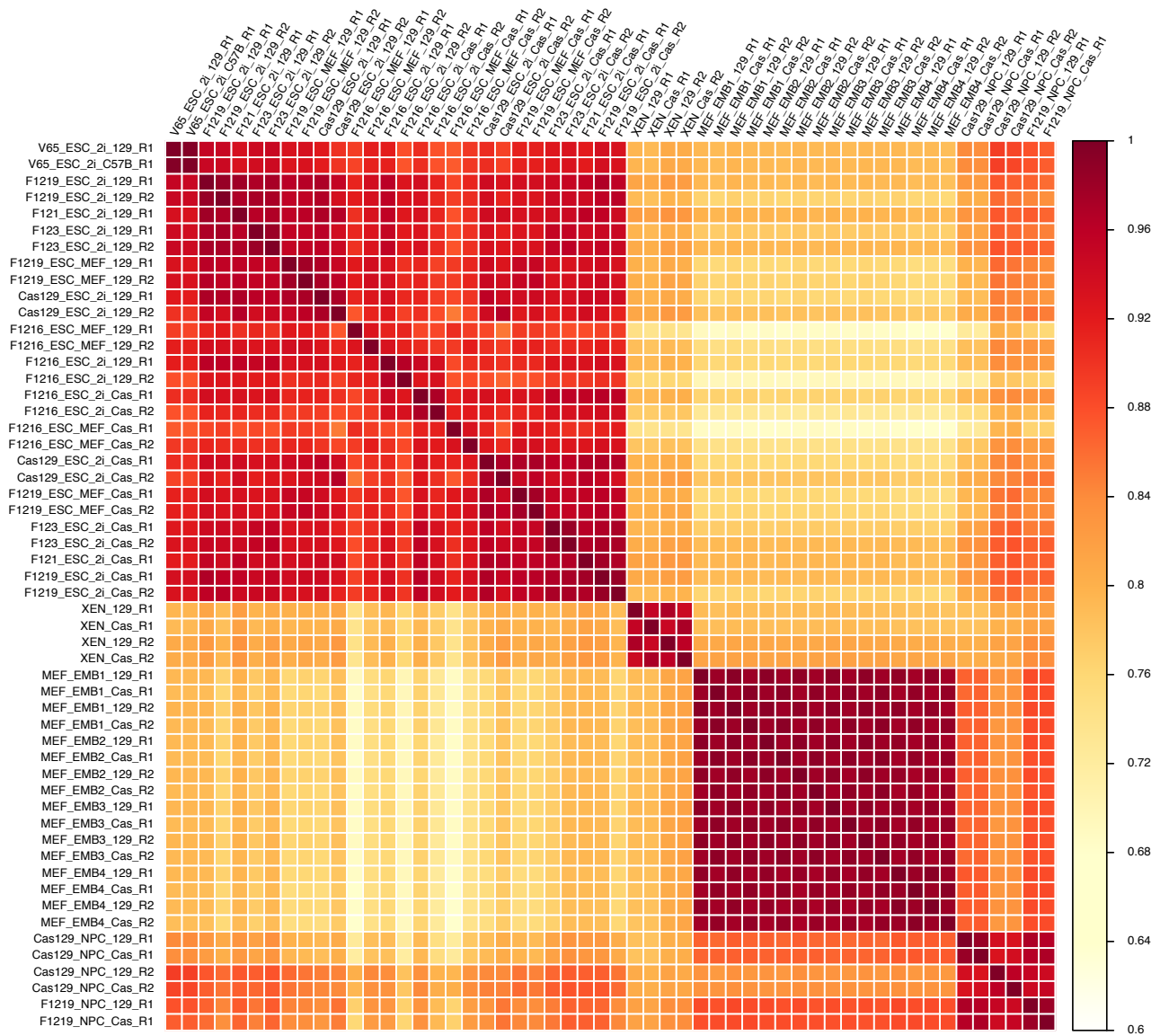


Supplemental Figure S9. RT profiles of the chromosome X in distinct cell types derived from hybrid mouse crosses.



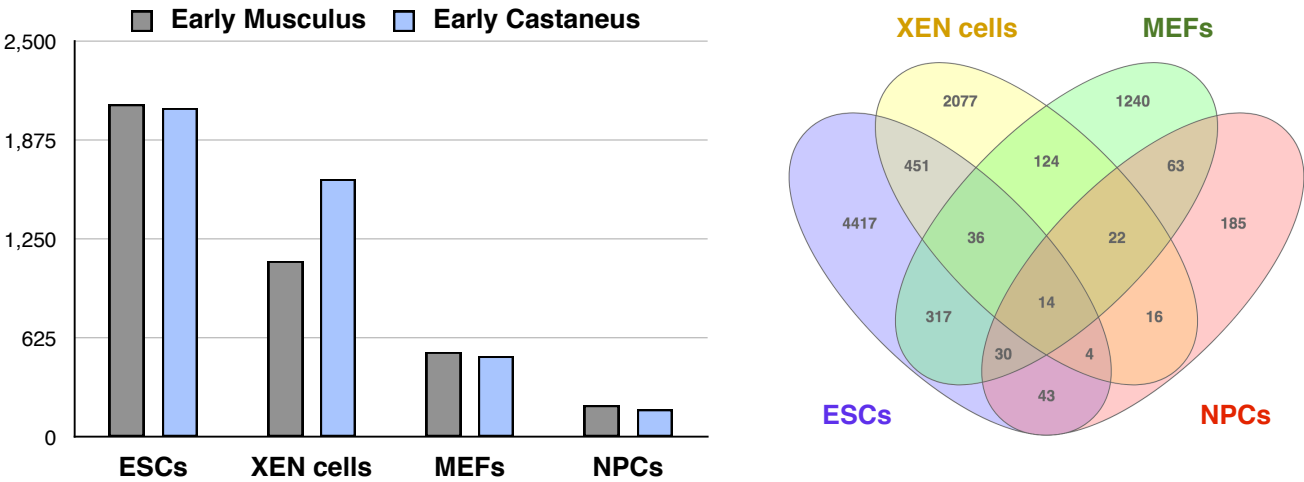
Supplemental Figure S10. RT asynchrony is linked to long-range enhancer-promoter interactions.

Long-range enhancer interactions are restricted to the allele that replicates earlier. In the two exemplary regions at the top long-range interactions connecting the asynchronous domain with other distant early replication domains are restricted to the *musculus* allele (grey), while the regions at the bottom show long-range interactions restricted to the *castaneus* allele (blue).



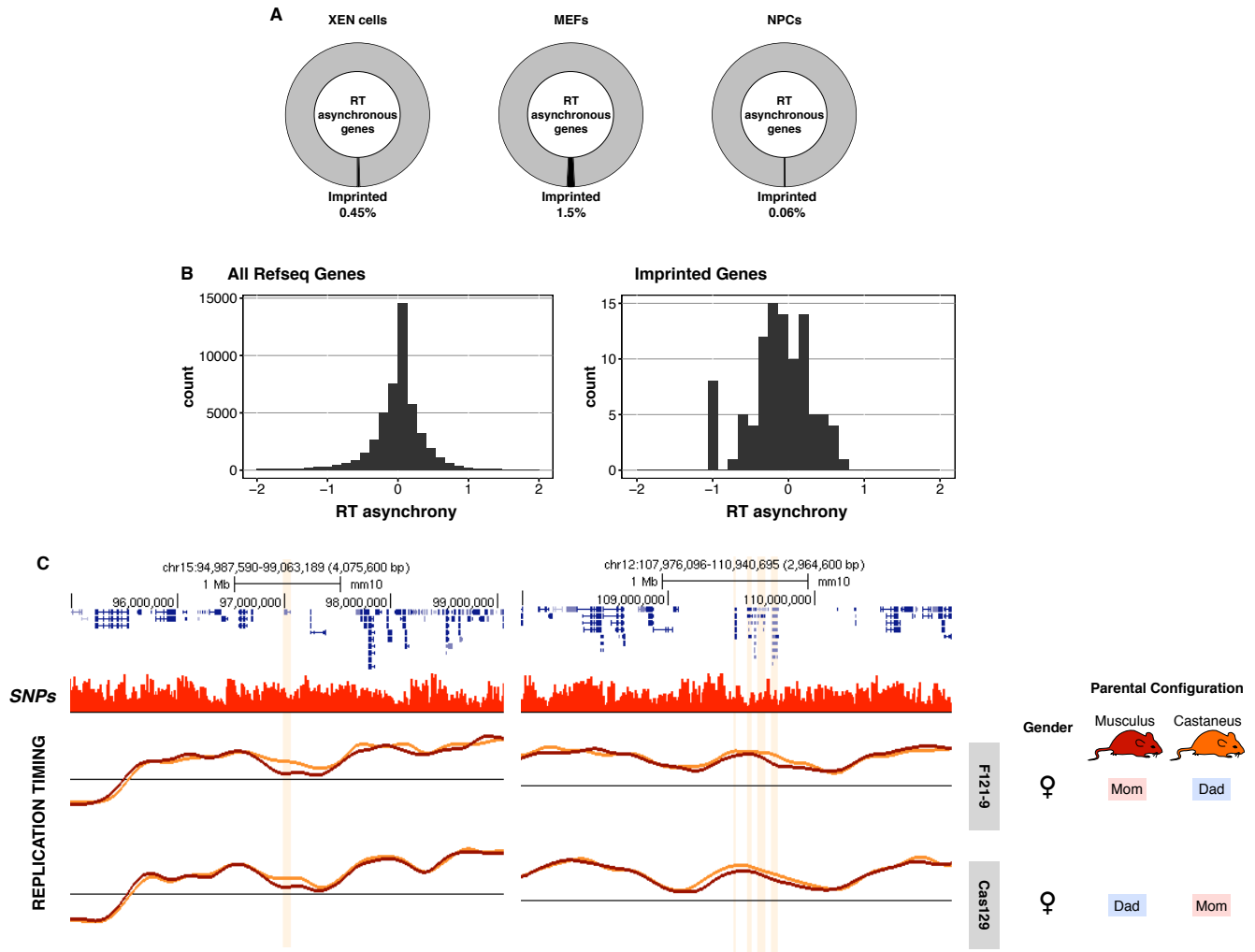
Supplemental Figure S11. Correlation matrix of RT programs from all hybrid mouse cell types.

Genome-wide correlation of RT programs from all hybrid mouse cell types including ES cells, XEN cells, MEFs and NPCs confirms that RT is cell type specific. Consistent with loss of RT asynchrony upon cell fate commitment, stronger correlations between alleles, replicates and cell lines were observed in all differentiated cells as compared to ESCs.



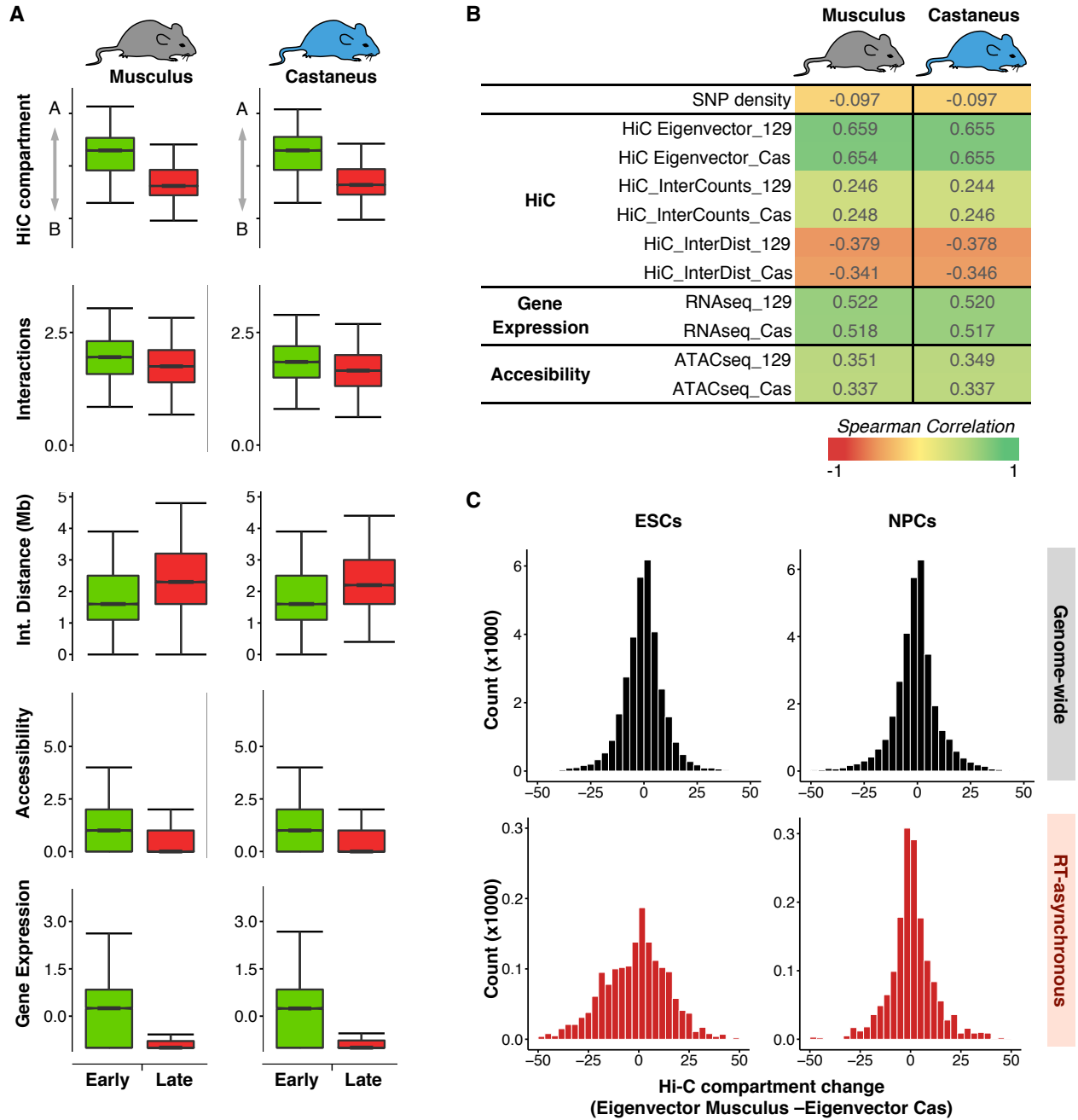
Supplemental Figure S12. Distinct regions replicate asynchronously in each hybrid mouse cell type.

Counts of RT asynchronous regions and overlap analysis in all hybrid mouse cell types.



Supplemental Figure S13. RT asynchrony is not associated with gene imprinting in differentiated cells.

(A) RT asynchronous genes and gene imprinting in differentiated cell types. RT values at the transcription start sites (TSS) of all RefSeq genes were obtained and asynchronous genes identified. RT asynchronous genes are not associated with gene imprinting. (B) RT asynchrony across all RefSeq genes and for imprinted genes in NPCs. (C) Exemplary RT profiles of the 2 imprinted loci with the highest RT differences in NPCs derived from hybrid mES cell lines with opposite parental configuration.



Supplemental Figure S14. RT and genome organization in NPCs.

(A)) Genome organization, chromatin accessibility and gene expression of RT synchronous regions that replicate either early or late during S-phase in NPCs. (B) Spearman correlation values of RT and distinct genomic features per genome. (C) Histograms of Hi-C eigenvector differences between genomes in hybrid ESCs and NPCs. Top panels show the differences genome-wide, bottom panels show the differences for the regions that replicate asynchronously in ESCs.

Cell line	V6.5	F121	F121-6	F121-9	Cas129	F123
DMEM	200 mL	500 mL	500 mL	-	500 mL	500 mL
GMEM	-	-	-	500 mL	-	-
FBS	50 mL	50 mL	75 mL	50 mL	75 mL	-
KOSR	-	-	-	-	-	50 mL
100x NEAA	3 mL	5 mL	5 mL	5 mL	5 mL	6 mL
100x Pyruvate	-	-	-	5 mL	-	-
100x Glutamine (200 mM)	3 mL	5 mL	5 mL	5 mL	-	-
100x nucleosides*	3 mL	-	-	-	-	-
100x Glutamax	-	-	-	-	-	6 mL
BME (FINAL)	1 mM	2.85 mM	5 mM	0.1 mM	2.85 mM	0.1 mM
100x Pen/Strept	3 mL	5 mL	5 mL	-	5 mL	6 mL
LIF	10 ³ U/mL	10 ³ U/mL	10 ³ U/mL	10 ³ U/mL	10 ³ U/mL	10 ³ U/mL
H2O	37.5 mL	-	-	-	-	-

Supplemental Table 1. ES media composition

*Millipore ES-008-D